

## CHAPTER III



### EXPERIMENTAL PROCEDURE

The aim of this study involves an attempt to improve the mechanical properties of porous HA by adding additives to the HA. We have also investigated the influence of additive content, with the intention of optimizing the mechanical properties and bioactivity. Therefore, this chapter consists of two major parts; (i) preparation of HA with varied amount of additives (commercial SiO<sub>2</sub> powder and SiO<sub>2</sub> in form of glass frit), (ii) characterization of the prepared HA with and without additives on microstructural, mechanical, and bioactive properties. The fabricated processes used were both of uniaxial pressing and polymeric sponge methods. Details of each part are described below.

#### 3.1 Starting Materials

The starting calcium phosphate powder with a fully crystallized structure of hydroxyapatite (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>) was purchased from a UK manufacturing company. The crystallinity of HA used in the preparation corresponded to a hydroxyapatite phase (JCPDS 09-0432). No additional phase could be identified from XRD analysis. The bulk characteristics characterized by X-ray fluorescence analysis indicated that the powder contained only minor amounts of compounds other than HA, shown in Table 3.1. The particle size distribution of HA powder was determined using laser particle size analysis as given in figure 3.1. The average particle size ( $d_{50}$ ) of HA particle was 5.85  $\mu\text{m}$ . The size distribution of HA powder was wide,  $d_{10} = 0.45 \mu\text{m}$  and  $d_{90} = 24.46 \mu\text{m}$ .

Table 3.1 Composition of hydroxyapatite as the raw material

oxides	CaO	P <sub>2</sub> O <sub>5</sub>	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	MgO	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	K <sub>2</sub> O	MnO
HA-130	55.94	43.30	0.34	0.05	0.28	0.01	0.04	0.01	0.02

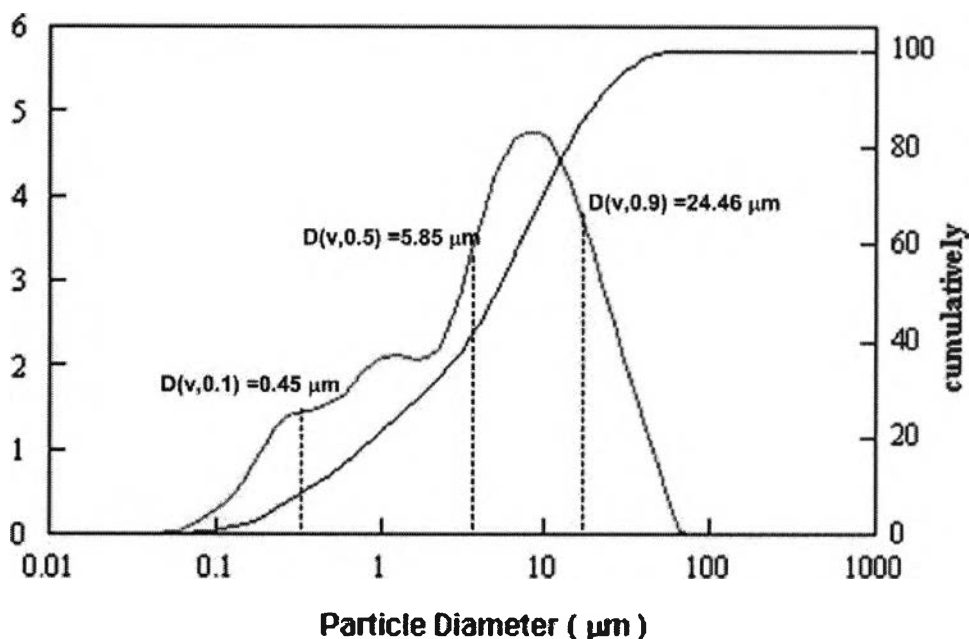


Figure 3.1 Size and particle size distribution of hydroxyapatite powder.

Commercially available silica (SiO<sub>2</sub>) nanopowder (Aerosil-200, Degussa Co.ltd.,Thailand) having average particle size of 12 nm, specific surface area of  $200 \pm 25 \text{ m}^2/\text{g}$  was used as an additive. Commercially available glass frit, coded no. 7406, was donated by Colorobia Co. Ltd., Thailand was also used as another additive to compare to the silica additive. The chemical composition is shown in Table 3.2. Dispex A 40 (ammonium polyacrylate, Ciba, UK) was added as a dispersant to control the slip viscosity without decreasing the solid loading. Moreover, polyethylene glycol (PEG), methyl cellulose, polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), used in this study are commercially available from the Sigma-Aldrich, Inc.

Table 3.2 Chemical composition of glass frit (Frit no. 7406) powder

oxides	SiO <sub>2</sub>	CaO	ZnO	Al <sub>2</sub> O <sub>3</sub>	K <sub>2</sub> O	B <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	TiO <sub>2</sub>	ZrO <sub>2</sub>
Frit No.7406	64.10	15.06	9.46	4.33	2.60	3.85	0.29	0.15	0.15

**Remark:** ignition loss is C<sub>6</sub>H<sub>8</sub>O<sub>3</sub>N<sub>2</sub> as binder for XRF sample preparation

### 3.2 Preparation of Porous Hydroxyapatite for Bone Replacement Applications

#### 3.2.1 Preparation of Porous HA with and without Additive by Uniaxial Pressing

The HA powder and additives, the silica or glass frit were mixed and ground in a ball mill using water as the suspension medium. Zirconia balls were used as the grinding media. Premixed suspensions were prepared which consisted of small amounts of additive to serve the functions of dispersant, plasticizer, surfactant and deformer, by dissolving in DI-water. The slurries were mixed and ground using ball milling technique and then dried in an electric oven. The dried powders were ground, sieved, and then pressed in a stainless steel mold having a rectangular shape, 13 mm × 35 mm, under a pressure of 14 MPa using a press machine from Carver, Inc (Model: M s/w 12000-132, USA). The green samples were sintered in an electric furnace and were heated to 650 °C with a heating rate of 1 °C/min and held at this temperature for 120 min. It was then raised to the maximum sintering temperature of 1150 and 1300 °C with a heating rate of 2 °C/min and held for 240 min. All samples were cooled in the furnace at a cooling rate of 2 °C/min.

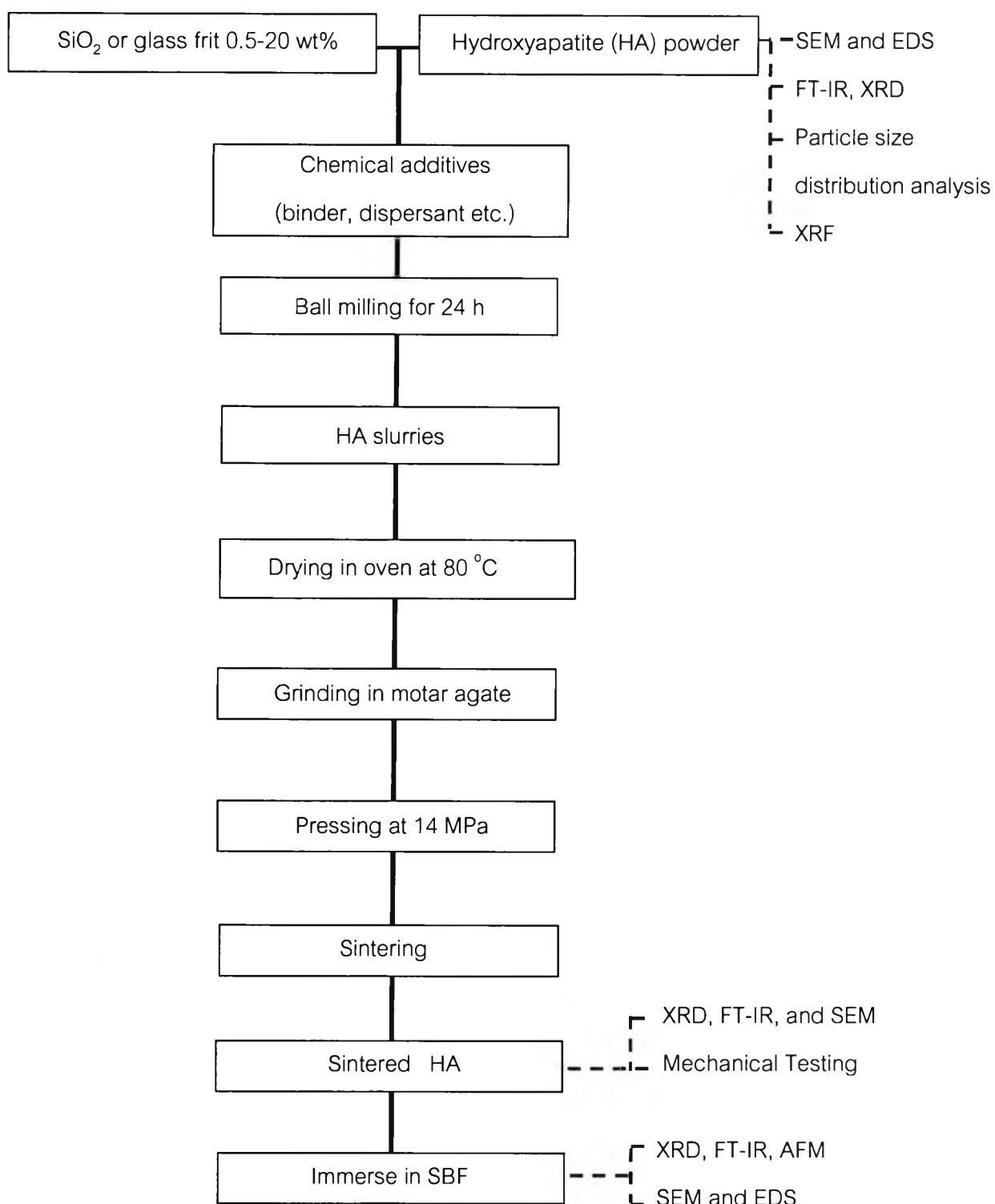


Figure 3.2 Flowchart of the porous hydroxyapatite fabricated by uniaxial pressing method.

### 3.2.2 Preparation of Porous HA with and without Additives by Polymeric Sponge Method

Pieces of polyurethane foams ( $20 \times 40 \times 20 \text{ mm}^3$ ), with a pore size about  $700 \mu\text{m}$  were cut from a large polyurethane foam sheet. The PU foams were then cleaned with DI-water before drying in air. The porous HA with additives was fabricated by impregnating the struts of polyurethane foam with ceramic slurry. The slurry was composed of HA powder, silica or glass frit additive, polyethylene glycol (PEG), methyl cellulose, polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), and DI-water. The HA powder and additives were mixed and ground in a ball mill using water as the suspension medium. Zirconia balls were used as the grinding media. The additives were mixed in the solution as 0.5, 1.0, 3.0, 5.0, 10.0 and 20.0wt% of the HA powder. The polymer templates were immersed in the slurries, which subsequently infiltrated the porous structure and HA when the powder particles adhered to the surface of polymer. The excess ceramic slurries in the macropores of the foams were needed to be driven away by hand squeezing, blowing with air gun to disperse the slurries uniformly throughout the porous scaffolds, avoiding blockages of the pores by the ceramic slurries. The green porous bodies were first dried in an electric oven at  $80^\circ$  for 24 hour. After drying completely, the green bodies were sintered in an electric furnace and at temperatures of 1100, 1200, 1300 and  $1400^\circ\text{C}$ . It was reported that the polyurethane foam was completely burned out at approximately at  $550^\circ\text{C}$  [46]. Thus, to allow sufficient time for the complete burnout of the polymer in HA scaffolds before the sintering started, the heating rate was set to  $1^\circ\text{C}/\text{min}$  up to  $650^\circ\text{C}$  with a dwell time of 120 min and then raised to the maximum sintering temperature with a heating rate of  $2^\circ\text{C}/\text{min}$  and held for 240 min. Then all samples were cooled in the furnace at a cooling rate of  $2^\circ\text{C}/\text{min}$ .

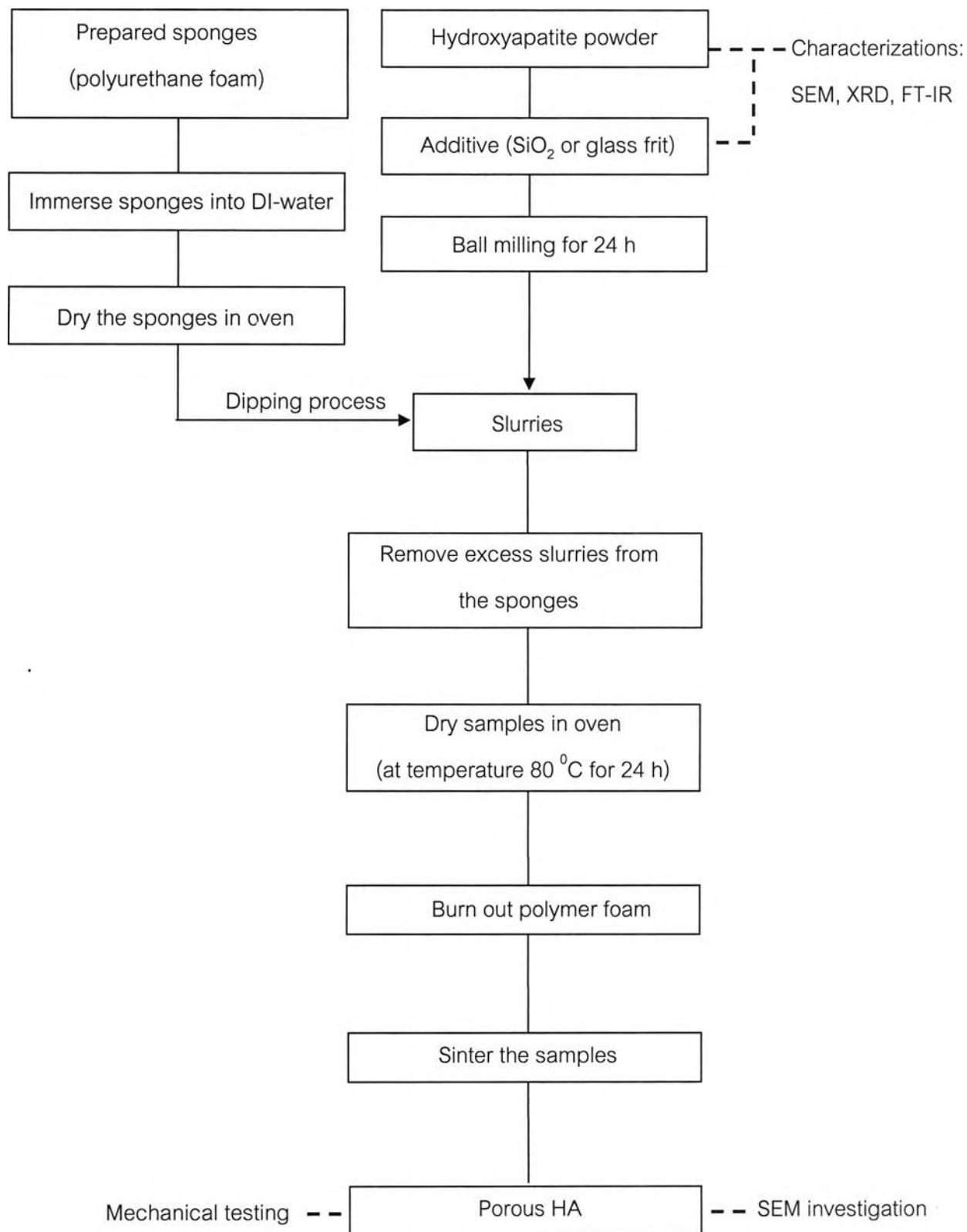


Figure 3.3 Flowchart of porous HA fabricated by polymeric sponge method

### 3.3 Characterizations

#### 3.3.1 Particle Size Distribution Analysis Using Mastersizer-S

The Mastersizer-S is basically a collimated He-Ne laser beam that passes through the sample and its dispersion measured. A small amount of powder was added to the dispersant and then ultrasonically mixed for 5 min. Then sample was poured into small volume unit with stirrer to pass the sample into laser unit. The scattered laser light from the sample is detected by the receiver of the optical measurement unit. This data from the receiver is transmitted to the computer system where the operating software calculates the size distribution.

#### 3.3.2 Phase Identification

The X-ray diffraction (XRD) technique is a widely used for studying structural information in condensed matter. The effect of the additives on phase transformation of the sintered samples were analyzed by X-ray diffractometry (XRD; Model: JEOL JDX-3530, Japan) using monochromated  $\text{CuK}\alpha$  radiation ( $\lambda = 1.5406 \text{ nm}$ ) produced at 30 kV and 40 mA. The diffraction angles ( $2\theta$ ) were scanned between  $5^\circ$  and  $80^\circ$  with a step size of  $0.04^\circ$  and step duration of 1.0 s. Identification of the phases was achieved by comparing the obtained diffraction patterns with a Joint Committee of Powder Diffraction Standards (JCPDS) database.

#### 3.3.3 Microstructural Analysis

A scanning electron microscope (SEM; Model: JEOL JSM-6301F) was used to observe and analyze the microstructure of the sintered structure to study and compare the influence of the additives on grain size, surface porosity and influence of additive

content on surface morphology evolution after immersion in simulated body fluid (SBF). The specimens to be observed were gold-coated for 100 seconds using a magnetron sputter coater (Model: JEOL JFC-1200). Energy dispersive analysis (EDS: Oxford, Inca 300) was used to investigate the elements of interest.

### 3.3.4 Chemical Composition Analysis

The X-Ray fluorescent (XRF, Philip PW-2404) analysis was performed to analyze the chemical composition of the as-received hydroxyapatite and glass frit powder. 5 g. of the HA or glass frit powder was mixed with 1 g. of tablet wax ( $C_6H_8O_3N_2$ ) used as binder. The weight ratio of the samples to the wax was about 5:1. The mixed powders were pressed under a pressure of 35 MPa to form a pellet 30 mm in diameter.

### 3.3.5 Chemically Functional Groups Analysis

Transmission infrared spectroscopy was performed by the KBr pellet method, using a Fourier Transmission Infrared Spectrometer (FT-IR; Model: Perkin Elmer system 2000, USA) to determine the chemically functional groups in the specimen powder of HA with and without additives. The powder was ground in an agate mortar and thoroughly mixed with spectroscopic grade KBr. Transparent pellets were prepared in a stainless steel die. FT-IR spectra were collected in the  $4000-400\text{ cm}^{-1}$  wavenumber range.

### 3.3.6 Porosity and Density Measurement

As-prepared powders with varied amount of additive were uniaxially compacted in a press mold made of stainless steel at a pressure of 14 MPa. The



apparent porosity and density of sintered samples were determined by Archimedes principle. Dry weight (D) was measured by a precision balance (Metler-Toledo AG 204, Thailand). The specimen was then submerged in water, boiled at 100 °C for 5 h and soaked for another 24 h. Then, the weight of specimen suspended in water (S) was measured. The specimen was taken out and blotted by tissue paper to remove the excess water and immediately reweighed (M). The porosity and density were calculated by the following equation:

$$\text{Bulk density (g/cm}^3\text{)} = \frac{D}{V} \quad \dots\dots\dots(3.1)$$

$$\text{Apparent porosity (\%)} = \left( \frac{M - D}{M - S} \right) \times 100 \quad \dots\dots\dots(3.2)$$

The porosity and density of the porous body, fabricated by polymeric sponge method, was calculated by measuring their dimensions and weights. Measurements of the overall dimensions were taken using Vernier calipers. The porosity and density were calculated by following equation.

$$\text{Bulk density} = \text{Mass} / \text{Volume} \quad \dots\dots\dots(3.3)$$

### 3.3.7 Mechanical Testing

The compressive strength of the porous HA with and without additives was measured using an Instron mechanical tester (Model: Instron 55R4502, Germany) at a cross head speed of 0.5 mm/min with scaffolds of 2:1 height-to-width ratio, typically 10 mm diameter and 20 mm height. During the compressive strength tests, the stress and strain response of the specimen was monitored. Loaded samples were covered with a thin sponge layer to obtain uniform load distribution. Compressive strength of each sample was determined and the average for each composition was calculated and compared for analysis. Microhardness measurements were carried out on flat samples,

HA with and without additives fabricated by uniaxial pressing at 14 MPa, using Vicker's microhardness tester (Model: MHT-10 microhardness, Australia) at a fixed load of 400 g. Specimens used in hardness testing had average dimensions of 12 mm in diameter and 2.5 mm in thickness. Three samples of each composition type were tested for their hardness at ten different locations. The average of these reading was computed, reported and compared.

### 3.3.8 *In vitro* Bioactivity Using Simulated Body Fluid (SBF) Immersion Technique

Porous HA ceramic specimens for *in vitro* bioactivity testing in simulated body fluid (SBF) were prepared via uniaxial pressing in a compression mold followed by sintering at 1300 °C for 4 h. Specimen of pure HA and HA with varied amount of additives were prepared, sintered and immersed in a dynamic SBF solution for varied periods of time. The dissolution rates and the possible bioactivity of the HA with and without additive were investigated by immersing the samples in simulated body fluid at pH 7.41 for varied periods. The SBF solution was prepared according to the procedure described as originated by Kokubo et al. [33] SBF has been used widely by researchers to test bioactivity of materials, by examining the formation of an apatite layer on the surface of the materials under testing. The *in vitro* formation of an apatite layer on the surface of bone-grafting material can be reproduced in a cellular SBF which is prepared to have an ion concentration nearly equal to that of human blood plasma. The composition of SBF, in comparison with that of human blood plasma, is shown in Table 3.3. The specimens were soaked in 10 ml of SBF at 37 °C for varied periods and the solution was not refreshed during the experimental time. After immersion periods of 6, 12, 24 h, and 3, 5, 7, 14 days, the samples were rinsed three times with de-ionized water, and dried at 80°C in the electric oven. The surfaces of the products before and after soaking in SBF were observed using SEM and AFM. X-ray diffraction was also used to identify the phase formation of products after soaking in SBF. In order to evaluate the

element composition in the products after the soaking, energy dispersive X-ray spectroscopy (EDS) was used.

Table 3.3 Nominal ion concentrations of simulated body fluid (SBF) in comparison with that in human blood plasma

	Ion concentrations (mM)							
	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>
Human blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5